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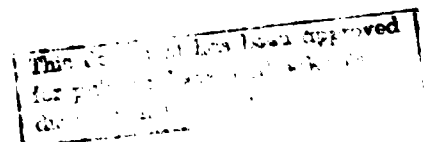
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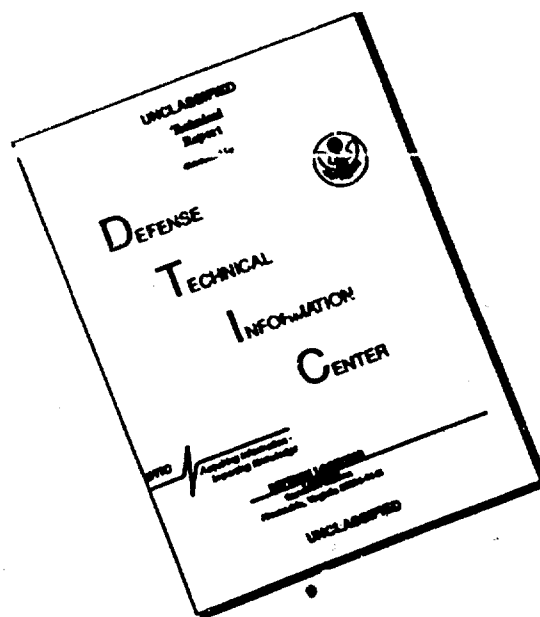
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A STUDY OF THE INTENSITY OF CROSS AND TYPE IMMUNITY IN BRUCELLOSIS

Following is a translation of an article by P.A. Vershilova and D.S. Kurdina of the Institute of Epidemiology and Microbiology imeni Gamaleya of the Academy of Medical Sciences of the USSR in the Russian-language periodical Zhurnal mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 8, 1963, pages 34-39. The article was submitted on 13 March 1962./

A large number of works, especially by Soviet authors, which have been devoted to the study of the nature of immunity in the case of brucellosis have confirmed the existence of cross immunity between types of Brucella organisms. This concept was decisive in selecting the cattle-type strain for vaccinating people against the infection caused by Br. melitensis. However, at the present time this question is undergoing a reexamination. Thus, the American researchers Herzberg, Elberg, Meyer, etc. (1953, 1955, 1958), while not denying the presence of cross immunity in brucellosis, consider that the type immunity has the greatest effect with respect to each type of Brucella organisms.

In our laboratory we have conducted work for many years in studying the properties and mechanisms of immunity in the case of brucellosis; therefore, we felt it necessary to perform additional research in order to study the type and cross immunity produced by vaccine cultures.

Tests were conducted on guinea pigs with the following

aspects under consideration. In the experimental study of the pathogenesis of brucellosis in animals of different kinds (sheep, cows, rabbits, guinea pigs, white mice, and rats) it was established that there is a species resistance to various *Brucella* types (*Br. melitensis*, *Br. abortus*, *Br. suis*). For example, the resistance of sheep to infection by cultures of *Br. abortus* and *Br. suis* is known. In order to produce infections in them with these types of *Brucella* organisms it is necessary to give considerably larger doses than when infecting with *Br. melitensis*. The species inertness of the organism to the given *Brucella* type with lowered virulence, i.e., to the vaccine strains, is found even more clearly than we observed in testing the vaccination of white mice with live *Br. abortus* culture (1954). Therefore, in order to study the type specificity of immunity upon vaccination with live vaccines it was necessary to use animals which were highly sensitive to all *Brucella* types. The guinea pig answered this requirement.

The tests were made on guinea pigs of one sex (males) and of the same weight (350-400 grams). The animals were immunized subcutaneously and were also infected subcutaneously in the right inguinal area. The infecting was conducted 45 days after vaccination; an autopsy was done 30 days after infecting.

For the bacteriological investigation lymph nodes were taken from each guinea pig (inguinal, paraaortal, neck, submaxillary); the same was done with the spleen, liver, bone marrow, blood, and urine. The immunological reactions were studied before infection and before the autopsy of the guinea pigs.

For the immunization laboratory vaccine cultures *Br. abortus* No 19-BA and 104-M and also *Br. melitensis* Rev. 1 obtained by Elberg (1955) were used. The immunogenic properties of the latter culture were studied by Elberg et al. in laboratory animals, monkeys, goats, and sheep. In our laboratory this strain was studied in detail by Kurdina (1961).

The typical antigenicity of the vaccine strains was determined using monospecific sera prepared in our laboratory under the control of internationally recognized brucellosis strains. Strains *Br. abortus* No 19-BA and 104-M and *Br. melitensis* were agglutinated with homological sera to a titer and were not agglutinated by heterological sera.

For the infection of vaccinated guinea pigs we used strains Br. melitensis No 565, Br. suis No 1330, and Br. abortus 10L which were agglutinated by homological monospecific sera to a titer.

Initially we intended to determine the intensity of cross immunity produced by strains of the type Br. abortus with various residual virulence (No 19-BA, and 104-M).

Test 1 was performed on 240 guinea pigs. The guinea pigs of the first group (120) were vaccinated with culture Br. abortus No 19-BA of 48-hour growth which was administered in an amount of one billion microbial cells (according to an enteric standard of turbidity); the guinea pigs of the second group (120 animals) received the same dose of culture Br. abortus 104-M. The large dose of vaccine strain 104-M was taken for immunization in order to provide equal test conditions, although it is known that this strain provides immunity of a high intensity with a smaller dose.

At 45 days after vaccination the guinea pigs of the first and second groups were divided into two sub groups; some were infected with the highly virulent culture Br. melitensis while others were infected with Br. suis. The infection of the guinea pigs was performed with 2, 5, 25, and 250 infecting doses (ID). There were 15 guinea pigs for each dose. One infecting dose for strain Br. melitensis was equal to 10-12 colonies grown in agar upon sowing 0.1 ml from the dilution of 10^{-8} of a suspension of a density of one billion (with respect to standard turbidity). For the strain Br. suis the corresponding dose was equal to 5-7 colonies produced in agar under the same conditions. In accordance with our determination, one infecting dose upon subcutaneous infection and opening the guinea pigs after 30 days caused a generalized infection with a serination coefficient with Brucella organisms for the organs of the animal within a range of 60-80 and higher.

Despite the relative reliability of the figures for the small number of test animals, it was nevertheless possible to see (Table 1) that strain Br. abortus 104-M produced immunity in guinea pigs to Br. melitensis and suis of a higher intensity than did strain No 19-BA. This difference could not be established only in the infecting of the animals with 2 infecting doses of culture Br. melitensis. The resistance of the vaccinated guinea pigs

of both groups to infection by 25 and 250 infecting doses of Br. suis was lower than with respect to infection by Br. melitensis, despite the fact that Br. suis No 1330 is not different in its antigenic structure from Br. abortus. We explain the lesser resistance of the guinea pigs to strain Br. suis No 1330 as being the result of its high toxigenicity and the suppression of defensive mechanisms in the vaccinated animal.

Table 1

The intensity of cross immunity in guinea pigs immunized with Br. abortus

Vaccine strain	Strain of infection	Number of uninfected guinea pigs, where they had received various numbers of infecting doses			
		2	5	25	250
Br. abortus No 19-BA	Br. melitensis	100	74	80	39
	Br. suis	79	72	50	15
No 104-M	Br. melitensis	100	93	93	54
	Br. suis	100	93	77	29

It should be noted that the defense of the organism of guinea pigs vaccinated with Br. abortus No 104-M was accomplished at 64-95% (by groups) non-sterile immunity, i.e., at the moment of opening (75 days after vaccination) the vaccine culture was isolated from the guinea pigs in the indicated percent. Guinea pigs vaccinated with strain No 19-BA at this time had sterile immunity for 87-100%.

Thus, the data of Test I showed that the level of the immunity caused by live vaccine of type Br. abortus with respect to virulent cultures of Br. melitensis and Br. suis was determined by the strength of antigenic stimulation and, in the given case, by the duration and intensity of the settlement of the vaccine culture in the organs of the animal.

Test II was devoted to a study of the intensity of type and cross immunity produced by vaccine strains which differ in their antigenic structure -- Br. abortus No 104-M and Br. melitensis Rev. 1.

The scheme and the method of the test were the same as for Test I. The guinea pigs of the first group were vaccinated with culture No 104-M with a dose of 100,000 microbial cells; the guinea pigs of the second group (168) received the same size dose of culture Rev. 1. At 45 days after vaccination the animals were divided into three equal groups and were infected with 2, 5, 25, and 250 infecting doses of Br. melitensis No 565, Br. suis No 1330, and Br. abortus 10L (one infecting dose of strain 10L was equal to 10-12 colonies grown in agar under the above indicated conditions). At 30 days after infection the guinea pigs were opened and a bacteriological study was made of the animals (Tables 2 and 3).

The differentiation of the cultures isolated upon bacteriological investigation of guinea pigs vaccinated with Br. abortus No 104-M and infected with a culture of the same type of 10L was performed based on the formation of hydrogen sulfide. Strain No 104-M does not form hydrogen sulfide upon growing in agar, while strain 10L discharges it actively. Cultures isolated from guinea pigs vaccinated with Br. melitensis Rev. 1 and infected with Br. melitensis were differentiated according to aniline dyes. The reducing capacity (fuchsin and thionine) of culture Rev. 1 was very weak.

The percent relationship of the positive secretions from the organs to the total amount of secretion produced from the entire group of guinea pigs was expressed by the group coefficient of infectivity of the organs of test guinea pigs which had been examined bacteriologically after infection. From each guinea pig 13 objects were examined. At the moment of investigation there were 13-16 guinea pigs in each group. Consequently, from the coefficient infectivity it was possible to compile a

sufficiently correct concept of the immunity of the vaccinated guinea pigs.

Table 2

The intensity of type and cross immunity in guinea pigs vaccinated with Br. abortus No 104-M and Br. melitensis Rev. 1

Vaccine strain	Infecting strain	Number of uninfected guinea pigs upon administering various numbers of infecting doses to them			
		2	5	25	250
Br. abortus № 104-M	Br. melitensis	94	81,3	81,3	56,3
	Br. suis	93	78,6	Not investigated	57,2
	Br. abortus	93	84,6	64,2	64,2
Br. melitensis Rev. 1	Br. melitensis	100	100	75	50
	Br. suis	100	93	54	42
	Br. abortus	100	94	94	59

However, the numerical data which we obtained was subjected to additional statistical processing according to the formula for the average error of the proportional index

$$\sqrt{m} = \sqrt{\frac{P(100-P)}{n}}$$

and we calculated the limits

of the fluctuations of the calculated coefficients according to the formula

$$\sqrt{t} = \frac{P_1 - P_2}{\sqrt{m_1^2 - m_2^2}}$$

Table 3

Coefficient of the infectivity of the organs of guinea pigs by Brucella organisms where the guinea pigs have been vaccinated with Br. abortus No 104-M and Br. melitensis Rev. 1 and infected with various doses of Br. melitensis, suis and abortus

Number of infecting doses	Vaccine strain	Coefficient of infectivity upon infection with various doses					
		Br. melitensis		Br. suis		Br. abortus	
		Test	Control	Test	Control	Test	Control
2	<u>No 104-M</u> Rev 1	$\frac{0.5}{0}$	80	$\frac{1.7}{0}$	73	$\frac{0.7}{0}$	64
5	<u>No 104-M</u> Rev 1	$\frac{4}{0}$	80	$\frac{9}{4.4}$	91	$\frac{3}{0.6}$	80
25	<u>No 104-M</u> Rev 1	$\frac{2.4}{2}$	82	Not investigated $\frac{18}{18}$	91	$\frac{6.4}{0.6}$	76
250	<u>No 104-M</u> Rev 1	$\frac{11.3}{16.2}$	83	$\frac{12}{19.3}$	87	$\frac{6.5}{4.1}$	Not investigated

Based on the results of Test I and II we feel that it is possible to make a series of conclusions.

Thus, vaccine strains Br. melitensis Rev. 1 and abortus No 104-M are close with respect to residual virulence (Kurdina, 1961); they produced immunity of equal force. The vaccinated guinea pigs displayed less resistance to infection by the highly virulent culture Br. suis than

to infection by strains Br. melitensis and abortus. Consequently, the immunity produced by strain Br. abortus No 104-M which was close in its antigenic structure to Br. suis did not have an advantage.

Cross immunity produced by live vaccines when tested for its intensity in the non-sterile phase was not inferior to type immunity. Data on the study of the intensity of type and cross immunity in the sterile phase will be presented by us in a subsequent work.

Thus, the results of our present and past works (Vershilova, 1961) provide a basis for the utilization of Brucella strains of various types for vaccinating human beings and animals. However, if we consider the different species sensitivity of the organism to equivalent Brucella types, as we see it the selection of vaccine strains should be performed with consideration for the following basic concepts. In vaccinating sheep or goats one should employ cattle-type vaccine strains with higher residual virulence than the strains used in vaccinating cattle. This is necessary in order to overcome the species immunological inertness of the organism of sheep to Brucella organisms of the cattle-type. Proof of this is found in the tests by Abakin, Zamakhayeva, and Chernysheva (1962) who worked with sheep. The authors showed that strain Br. abortus No 104-M with its higher residual virulence provided better immunity with respect to Br. melitensis than strain No 19-BA. If we turn to the question of selecting a strain for the vaccination of human beings, then it is necessary to consider the fact that man is less resistant to infection by strains of the type Br. abortus than are sheep and goats. Consequently, in selecting the strains for vaccinating human beings with live vaccine, it is necessary first of all to select a strain which would be safe and would not cause the disease, but would be highly immunogenic and provide protection for the organism against Brucella organisms of the goat-sheep type. With respect to the necessity of finding Brucella vaccine strains of the melitensis type for vaccinating human beings and sheep, this problem has been posed for solution.

We have not given detailed information on the study of immunological reactions, inasmuch as they do not permit a determination of the intensity of immunity. Let us only mention that the immunological rebuilding at 45 days after vaccination was more expressed in guinea pigs which had been vaccinated with Br. abortus No 104-M

(Test II). The titer of the agglutination reaction in these guinea pigs was 3-4 times higher than in guinea pigs vaccinated with strain Rev. 1; the allergic skin test was positive in 77% of the cases whereas with guinea pigs which were vaccinated with Rev. 1 it was positive in 67% of the cases.

Conclusions

1. Investigations which were made with guinea pigs to test the intensity of type and cross immunity produced by live vaccines of the type Br. abortus (No 104-M, No 19-BA) and melitensis (Elberg's strain Rev. 1) with respect to infection by highly virulent cultures of Br. melitensis (No 565), Br. suis (No 1330), and Br. abortus (10L) do not provide a basis for indicating that cross immunity is weaker than type immunity in the case of brucellosis.

2. In a comparative study of the immunogenicity of vaccine strains of the cattle and sheep types under conditions of testing the intensity of immunity by various doses of highly virulent cultures of Br. melitensis, suis and abortus, it was established that strain Rev. 1 has a high immunogenicity which is not inferior to that of Br. abortus No 104-M.

3. Strain Br. abortus No 104-M upon subcutaneous vaccination produced immunity in guinea pigs to cultures of Br. abortus, suis, and melitensis which were more intense than was the case with strain Br. abortus No 19-BA.

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